ESTIMATION OF ANT COLONY SIZE BY THE LINCOLN INDEX METHOD[1]

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Apparently the only method used in the past for estimating the size of ant colonies has been to excavate the colony completely and count all individuals unearthed. The uncertainty and difficulty of this method is well illustrated by the history of *Myrmecocystus melliger* Forel. Wheeler (1908) felt that this species forms small colonies of hardly more than 300–500 individuals with chambers that do not descend more than a foot or so into the soil. He thought that he had completely excavated such colonies. That this may not have been true is obvious from the report of Creighton and Crandall (1954) on the excavation of one colony near Tucson. At the beginning of excavation, the vertical extent of this colony was apparently going to be limited by a rock-like layer of caliche, eighteen inches below the soil surface. However, careful following of a single small lead descending vertically through the caliche led to the discovery of new chambers at a depth of thirty inches. After penetration of five to six feet of caliche, the excavation was completed at a depth of sixteen feet, where the queen's chamber was found. Over 1500 repletes and hundreds of normal workers were found in the many chambers of this colony. Previously this species was thought not to have repletes. Tevis (1958) followed colonies of *Veromessor pergandei* (Mayr) as deeply as eleven feet without completely excavating any colony.

While certain biological information, such as the occurrence of repletes in *M. melliger,* can be obtained only by laborious digging, the present author has found that the number of foraging workers can be estimated much more simply, by the use of the Lincoln Index method, or the mark-release-recapture method. This method has been widely used in censusing populations of different vertebrate animals.

As used by the author, this method is as follows: (1) 100–400 worker ants are collected from a colony entrance with an aspirator; (2) these individuals are etherized in several batches and each ant is marked on the dorsum with a spot of Testors colored

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dope; (3) the marked ants are counted and then released at the colony entrance after they have revived (the few ants whose legs have become bound up by dried dope are removed); (4) a second sample of ants is collected 24 hours later, and the marked and unmarked individuals counted; (5) the total number of foraging workers in the colony is calculated by the formula:

$$\text{total no. workers} = \frac{(\text{No. ants in 2nd sample}) \times (\text{total no. marked})}{\text{Number of marked ants recaptured}}.$$

The use of this method is based on several assumptions: (a) any individual in the colony is susceptible of being captured and marked; (b) the marked individuals mix thoroughly with unmarked before resampling; (c) marking is permanent during the length of the sampling period, and does not adversely influence the behavior or survival of marked individuals. Considering these assumptions with regard to ants:

(a) As far as is known, all normal worker ants that are beyond the callow stage participate in foraging and carrying materials out of the nest. The situation in bees, where younger workers limit themselves to duties inside the nest, while only older workers forage outside, is not known to occur in ants (W. S. Creighton, personal communication). Therefore, any individual worker is susceptible of being captured outside the nest and marked or counted.

Certain special types of workers do not normally leave the nest, and these would not be captured or enter into the estimation of colony size. Repletes, such as found in some species of the genus *Myrmecocystus,* do not leave the nest, but full repletes are not found in any other North American genus. The major workers of many species of *Pheidole* do not forage, and the majors of *Cryptocerus* and *Camponotus* probably do not forage. These exceptions are few and they do not limit the application of the Lincoln Index method to most species of ants.

(b) The thoroughness with which marked and unmarked workers mix within the colony in 24 hours is not yet certain. When there are several openings to a colony, several feet apart, marked individuals released at one entrance are not necessarily captured in equal ratios at all entrances 24 hours later. This indicates incomplete mixing.

(c) Spots of Testors colored dope are not permanent markings. The dope flakes off in a matter of one to several days.

Counting of marked individuals in the recapture sample is best done under magnification, in order to detect those ants that have only a small fleck of dope still adhering to a hair or bristle. When a colony is resampled at 24-hour intervals, the calculated colony size shows a gradual increase. This indicates inversely the rate at which marked individuals are losing their identification. If such data are plotted, as in Figure 1, the curve can be interpolated to zero time, i.e. no loss of markings, for a possibly more accurate estimation of colony size. The loss of markings in the first 24 hours for *Pogonomyrmex occidentalis* is within the range of variability of different samples taken at 24 hours after release. It should be possible to find a more adherent marking material, which would allow a sampling interval of more than 24 hours and thus more thorough mixing in of marked individuals.

There is no evidence that this method of marking results in the death of marked individuals.

Table 1 gives preliminary data on colony size of three species of ants in Cochise County, Arizona. As expected, there is a

TABLE 1.

NUMBER OF FORAGING WORKERS IN COLONIES OF THREE SPECIES OF ANTS IN COCHISE CO., ARIZONA.

Colony #	Date	Foraging Workers	Mound Size
Novomessor cockerelli (E. Andre)			
A13	Oct. 3	236	52 cu. in.*
A8	Oct. 13	570	254 cu. in.
A4	Oct. 13	648	221 cu. in.
A1a	Oct. 13	570	—
Myrmecocystus mimicus Wheeler			
A25	Oct. 22	650	39 sq. in.**
A26	Oct. 23	1280	29 sq. in.
Pogonomyrmex occidentalis (Cresson)			
P1	Sept. 21	1320	302 cu. in.*
P2	Sept. 21	435	74 cu. in.
P3	Oct. 8	2100	1470 cu. in.

* mound size figured as volume of cone; ** basal area of crater-like mound.

N. cockerelli and *M. mimicus* colonies were located in a creosote bush community, 4500′ elevation, 6 mi. north of Portal, Arizona; *P. occidentalis* colonies were in pine-oak woodland, 5400′ Southwestern Research Station, Portal.

range of sizes for a particular species. A relationship between colony size and surface mound size is suggested.

The marking of ants with colored spots is also highly useful in determining whether adjacent mounds and entrances are parts of a single colony or separate colonies. Tèvis (1958) found that *Veromessor pergandei* colonies change their entrance hole about 10 times a year. One entrance is abandoned and another opened up. In the course of a year these different entrances of the same colony cover an area approximately 50 feet in diameter. Similar shifts of activity, and also simultaneous use of several entrances, have been observed by the

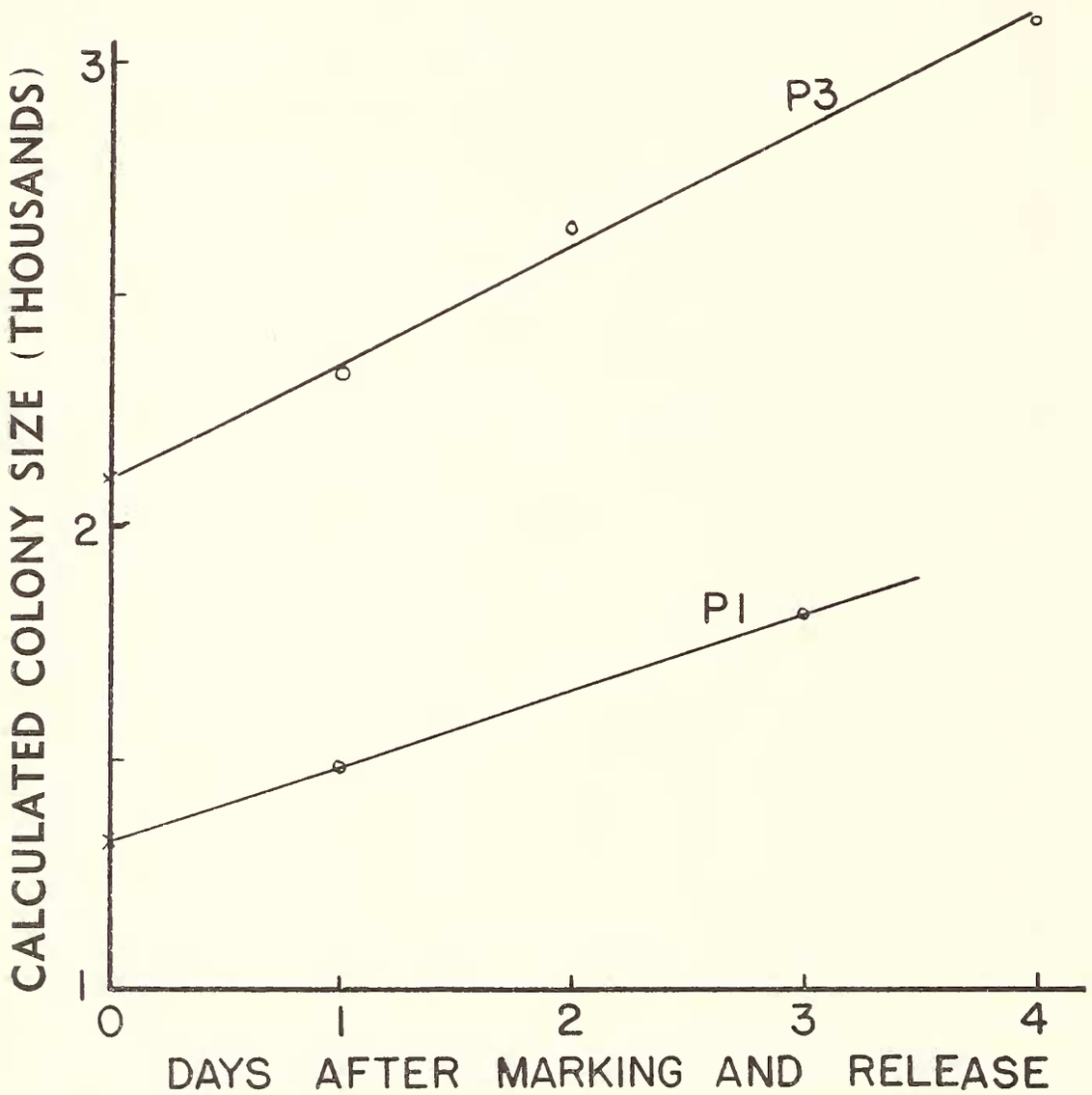

Figure 1. Estimation of number of foraging workers in colony of *Pogonomyrmex occidentalis*. True colony size is taken as value of line extrapolated to zero time, i.e. before any marking on ants had flaked off.

present author for *Novomessor cockerelli.* Colony A4 in Table 1 had three openings in a triangular arrangement 6 to 10 feet apart. Two of these openings had relatively high mounds, while the third was marked only by a circle of fine angular gravel. In the initial test to determine whether these openings were all part of a single colony, 100 individuals were marked green at entrance A, 118 were marked blue at entrance B, and none were marked at entrance C. Resampling at all entrances 24 hours later showed: A—115 unmarked, 45 green, 11 blue; B—62 unmarked, 49 blue, 2 green; C—48 unmarked, 4 blue, 3 green. While the three entrances are indicated as part of one colony, there was an obvious tendency for recapture of a particular color marking predominantly at the entrance where it was used.

The present paper is presented in the hope of stimulating further testing, development and use of the Lincoln Index method in ant work. Data easily obtained on size of colonies can be the bases for different kinds of ecological studies. The author is using the method to follow seasonal changes in colony sizes and as a basis for the calculation of the energy requirements of colonies, i.e. (colony size) × (measured metabolism of individual ants) = total energy requirement for colony. The method can also be used to follow growth of colonies from year to year. The mark-release-recapture method has the great advantage that it can be used repeatedly on the same colony, while the digging method destroys a colony at first use.

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